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Published:
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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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12 January 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: LIVE ATTENUATED NIDOVIRUS VACCINES

(57) Abstract: The present invention is directed live, attenuated Nidovirus vaccines, and in a particular embodiment, to coronavirus vaccines. The vaccine comprises a viral genome encoding a replicase polypprotein having at least one proteinase cleavage site that exhibits reduced or no cleavage. Such viruses show reduced

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/12441

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 39/12, 39/215, 39/29; C12N 7/00, 7/04
US CL : 424/204.1, 221.1, 225.1, 233.1; 435/235.1, 236
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/204.1, 221.1, 225.1, 233.1; 435/235.1, 236

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BONILLA et al. Characterization of the Leader Papain-like Proteinase of MHV-A59: Identification of a New in Vitro Cleavage Site. 1995, Vol. 209, pages 489-497, especially Table 1 and Figure 6.	1-4,9-15, 34 and 35
Y	VAN DINTEN et al. Proteolytic Processing of the Open Reading Frame 1b-Encoded Part of Arterivirus Replicase Is Mediated by nsp4 Serine Protease and is Essential for Virus Replication. March 1999,Vol. 73. No. 3, pages 2027-2037, especially Figures 2-6.	5-8, 16-33 and 36-40
Y	US 3,590,127 (BRYANS et al.) 29 June 1971 (29.06.1971), columns 2-6.	5-8, 16-33, 36-40
Y	SNIJDER et al. Primary Structure and Post-Translational Processing of the Berne Virus Peplomer Protein. October 1990, Vol. 178. No. 2, pages 355-363. Abstract Only.	5-8, 16-33 and 36-40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
17 September 2005 (17.09.2005)

Date of mailing of the international search report
04 NOV 2005

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/12441

Continuation of B. FIELDS SEARCHED Item 3:
US Pat, USPgPub, EPO, JPO, Derwent, medline, vetu
search terms: Nidovirus, Berne, Arteritis, Lelystad, PRRS, porcine respiratory and reproductive syndrome, murine hepatitis, cleavage, proteinase, delete, mutate, alter, coronavirus, attenuate, p28, p65, Breda, replicase

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference VBLT:038WO	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2004/012441	International filing date *(day/month/year)* 22 April 2004 (22.04.2004)	Priority date *(day/month/year)* 23 April 2003 (23.04.2003)]
International Patent Classification (IPC) or national classification and IPC [7] A61K 39/12, 39/215, 39/29, C12N 7/00, 7/04		
Applicant VANDERBILT UNIVERSITY		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 18 November 2005 (18.11.2005)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Nora Lindner
Facsimile No. +41 22 740 14 35	Telephone No. +41 22 338 89 65

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
STEVEN L. HIGHLANDER
FULBRIGHT & JAWORSKI, LLP
600 CONGRESS AVENUE, SUITE 2400
AUSTIN, TX 78701

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	04 NOV 2005
Applicant's or agent's file reference VBLT:038 WO	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/12441	22 April 2004 (22.04.2004)	23 April 2003 (23.04.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): A61K 39/12, 39/215, 39/29; C12N 7/00, 7/04 and US Cl.: 424/204.1, 221.1, 225.1, 233.1; 435/235.1, 236

Applicant

VANDERBILT UNIVERSITY

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☐ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	17 September 2005 (17.09.2005)	Shanon Foley Telephone No. (703) 308-0196

Form PCT/ISA/237 (cover sheet) (April 2005)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/12441

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into ____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ on paper

☐ in electronic form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/12441

Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 5-8, 16-33 and 36-40	YES
	Claims 1-4, 9-15, 34 and 35	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-40	NO
Industrial applicability (IA)	Claims 1-40	YES
	Claims NONE	NO

2. Citations and explanations:

Please See Continuation Sheet

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/12441

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:

Claims 1-4, 9-15, 34 and 35 lack novelty under PCT Article 33(2) as being anticipated by Bonilla et al. (Virology. 1995; 209: 489-497).

The claims are drawn to a live, attenuated virus encoding a replicase polyprotein with at least one cleavage site that exhibits reduced or no cleavage. The virus is a murine hepatitis virus that contains a deletion at cleavage site p28-p65.

Bonilla et al. anticipate murine hepatitis virus deletion mutants with reduced or no cleavage due to deletions at cleavage sites at p28 and p65, see the abstract, Table 1 and Figure 6.

Claims 6-8, 16-19, 21-33 and 36-40 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Van Dinten et al. (Journal of Virology. 1999; 73 (3): 21027-2037) and Bryans et al. (US 3,590,127).

The claims require that the virus is equine arteritis virus. The claims are also drawn to vaccine formulations and a method of inducing an immune response with a virus comprising deletions in at least one proteinase cleavage site.

See the teachings of Bonilla et al. above. Bonilla et al. do not teach an equine arteritis virus.

Van Dinten et al. teach mutatgenesis at multiple cleavage sites in equine arteritis virus that abolished protein processing and resulted in inhibition of infectious virus production, see the abstract and Figures 2-6.

Neither Van Dinten et al. nor Bonilla et al. teach a method of inducing an immune response.

However, Bryans et al. teach a method if inducing an immune response with an attenuated equine arteritis virus vaccine that is attenuated by serial passage, see claims 1-4.

One of ordinary skill in the art at the time the invention was made would have been motivated to administer the deletion mutant viruses of Bonilla et al. or Van Dinten et al. to induce an immune response against the virus, see column 2, lines 5-28 of Bryans et al. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of inducing an immune response with the deletion mutant of Bonilla et al. or Van Dinten et al. because Van Dinten et al. teach that analogous deletion mutations that negatively effect protein cleavage reduces the capacity to produce infectious progeny, see the last paragraph on page 2036, and Bryans et al. demonstrate that avirulent viruses comprising multiple mutations induce protective immune responses, see column 3, line 39 to column 5, line 21.

Alternatively, one of ordinary skill in the art at the time the invention was made would have been motivated to recombinantly incorporate the mutations of Van Dinten et al. or Bonilla et al. into the equine arteritis virus vaccine of Bryans et al. to reduce the possibility of wild-type reversion. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success for introducing the corresponding proteinase deletions of Bonilla et al. or Van Dinten et al. into the virus of Bryans et al. because Van Dinten et al. and Bryans et al. both teach attenuating measures for equine arteritis virus.

Claims 5and 20 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Snijder et al.(Virology. 1990; 178 (2): 355-363, abstract only).

The claims require that the virus is a Berne virus.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

See the teachings of Bonilla, Van Dinten and Bryans et al. above. None of the references teach a Berne virus.

However, Snijder et al. teach the post-translational processing of the Berne virus, see the abstract provided.

One of ordinary skill in the art at the time the invention was made would have been motivated to incorporate the corresponding deletion mutations to the proteinase cleavage sites of Bonilla et al. or Van Dinten et al. into the Berne virus of Snijder et al. to reduce the ability of the Berne virus to make infectious particles, see the last paragraph on page 2036 of Van Dinten et al. One of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of reducing infectious Berne virus progeny of Snijder et al. by introducing the corresponding deletion mutations of Bonilla et al. or Van Dinten et al. into the Berne virus of Snijder et al. because Van Dinten et al. teach that corresponding mutations have the same effect in all nidoviruses, see the previous citations, which is what the Berne virus is.

Therefore, the invention as a whole would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made, absent unexpected results to the contrary.